

# Acantholippia salsoloides: Phytochemical Composition and Biological Potential of a Thujonic Population

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## Abstract

*Acantholippia salsoloides* (Verbenaceae) is an aromatic plant widespread in the Andean region. The infusion (leaves and flowers) is widely used as a digestive stimulant as well as for the treatment of various diseases in traditional medicine. *A. salsoloides* attributes its common name “rica-rica” to the fresh and sweet fragrance of the plant. In this work, 2 different polar extracts and the essential oil of a selected rica-rica population were studied. The phenolic composition was analyzed by high-performance liquid chromatography diode array detector; the essential oil profile was determined by gas-chromatography ion-trap mass spectrometry/flame ionization detection. For all extracts, the antibacterial potential was performed by in vitro assays; the antioxidant and  $\alpha$ -glucosidase inhibition were determined in decoction and hydroethanolic extracts. The volatile profile allowed the identification of 26 volatile compounds,  $\beta$ -thujone (84%) being the major one in this rica-rica population. Eighteen phenolic compounds were identified; isoferulic acid (16%-18%) and cynaroside (45%-47%) were the larger ones. In a general way, the hydroethanolic extract was more active against *Staphylococcus aureus* and *Micrococcus luteus* (minimum inhibitory concentrations = 0.3–1.3 mg/mL). Both polar extracts have strong antiradical activities although decoction extract proved to be more active against DPPH<sup>•</sup> (half-maximal inhibitory concentration [IC<sub>50</sub>] = 36  $\mu$ g/mL) and O<sub>2</sub><sup>•-</sup> (IC<sub>50</sub> = 28  $\mu$ g/mL) while hydroethanolic extract shows higher action over  $\alpha$ -glucosidase (IC<sub>50</sub> = 217  $\mu$ g/mL). The results suggest that *A. salsoloides* leaves and flowers may be an interesting source of natural antioxidants, antidiabetics, or antimicrobials, and could be used in dietary supplements, medicinal products and pharmaceutical formulations.

## Keywords

*Acantholippia salsoloides*,  $\beta$ -thujone, isoferulic acid, cynaroside, biological action

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*Acantholippia salsoloides* Griseb. (Verbenaceae) is a spiny shrub popularly known as “rica-rica”<sup>1,2</sup>; synonyms are *A. hastulata* Griseb., *Lippia hastulata* (Griseb.) Hieron., and *Lippia salsoloides* (Griseb.) Briq. *A. salsoloides* attributes its common name rica-rica to the fresh and sweet fragrance of the plant.<sup>3–5</sup> The infusion of rica-rica (stems, leaves, and flowers) is used traditionally for the treatment of digestive infections, diarrhea, gastrointestinal bloating, dyspepsia, and to cure cold diseases.<sup>1,2,6</sup> Rica-rica leaves and flowers are widely consumed either alone or in combination with yerba-mate, as a digestive stimulant.<sup>7,8</sup>

The essential oil (EO) composition of *A. salsoloides* from several locations in the Andean region has been previously investigated showing significant diversity.<sup>6,9,10</sup> In Jujuy Province (Northwest of Argentina), the populations investigated showed different EO compositions too; in Chucalezna they accumulated mainly *p*-cymene (52.8 %), thymol (46.8 %), or  $\beta$ -thujone (63.3%-65.4%).<sup>6,8</sup> In other locations, the

main components were  $\beta$ -thujone (70.0%-72.5%), *trans*-sabinol, and *trans*-sabinyl acetate.<sup>6</sup> Regarding the biological potential of *Acantholippia*, previous studies include the repellent action of *A. salsoloides* and *A. seriphoides* EOs against *Aedes aegypti*<sup>9,10</sup>; the antifungal, antibacterial, and

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repellent effects of EOs of *A. seriphioides* and *A. deserticola* EOs<sup>1,2,11</sup> and the antimicrobial activity of hydroethanolic extracts (HEs) of *A. punensis* and *A. salsoloides*.<sup>7,12</sup> The aims of this study were to investigate *A. salsoloides* regarding the volatile profile of the EO and the phenolic compounds in the HE and decoction extract (WE), in addition to the antibacterial potential of these fractions. The antiradical activity and the  $\alpha$ -glucosidase inhibition were studied too, and related to the polar extracts composition.

The yield of EO obtained from the samples by distillation was  $0.9\% \pm 0.2\%$  v/w (average of 2 extractions on dry weight basis). Comparable results were previously reported for the EO extraction in *A. seriphioides* growing in Central Argentina.<sup>13</sup> The yields obtained with extraction procedures

were  $23.8\% \pm 1.4\%$  and  $21.8\% \pm 1.1\%$  (on dry weight basis) for HE and WE, respectively (and  $p$ -value  $> 0.05$ ).<sup>14,15</sup> In the EO of rica-rica growing in Chuzalezna, 26 components were identified and quantified, representing 97.9% of the oil (Table 1). The EO compounds present in a higher proportion were  $\beta$ -thujone (84.3%),  $\alpha$ -thujone (2.3%), limonene (2.7%), and sabinene (2.0%). These results are consistent with those obtained in previous studies.<sup>6</sup>

The high-performance liquid chromatography diode array detector (HPLC-DAD) analysis of extracts of rica-rica allowed the determination of 21 phenolic compounds. The identified compounds comprise 6 phenolic acids and derivatives (1, 3, 4, 7, 12, and 17) and 15 flavonoids (5-11, 13-16, and 18-21) (Table 2). Both polar fractions revealed similar

**Table 1.** Chemical Composition of *Acantholippia salsoloides* Essential Oil.

No.	Component <sup>a</sup>	RI <sup>b</sup>	RI <sup>c</sup>	t <sub>R</sub> <sup>d</sup>	(%) <sup>e</sup>
1	$\alpha$ -Thujene	924	930	6.3	0.1 $\pm$ 0.0
2	$\alpha$ -Pinene	936	939	6.6	0.4 $\pm$ 0.1
3	Sabinene	967	975	7.8	2.0 $\pm$ 0.3
4	$\beta$ -Pinene	980	979	8.4	1.1 $\pm$ 0.5
5	Myrcene	989	991	8.7	0.6 $\pm$ 0.1
6	$\alpha$ -Terpinene	1 015	1 017	9.7	0.1 $\pm$ 0.0
7	$p$ -Cymene	1 023	1 025	10.3	1.0 $\pm$ 0.2
8	Limonene	1 028	1 029	10.9	2.7 $\pm$ 0.2
9	1,8-Cineole	1 030	1 031	11.0	0.1 $\pm$ 0.0
10	$\gamma$ -Terpinene	1 060	1 060	12.3	0.4 $\pm$ 0.0
11	<i>cis</i> -Thujone ( $\alpha$ -Thujone)	1 103	1 102	12.8	2.3 $\pm$ 0.8
12	<i>trans</i> -Thujone ( $\beta$ -Thujone)	1 115	1 114	13.2	84.3 $\pm$ 1.1
13	Sabinol- <i>trans</i> (OH vs IPP)	1 145	1 142	13.4	0.2 $\pm$ 0.0
14	Thujanol < neoiso-3>	1 156	1 152	13.7	0.1 $\pm$ 0.0
15	Thujanol < neo-3>	1 158	1 154	14.0	tr <sup>f</sup>
16	Sabina ketone	1 162	1 159	14.8	tr
17	Terpinen-4-ol	1 179	1 177	15.3	0.4 $\pm$ 0.0
18	Thuj-3-en-10-al	1 188	1 184	15.6	tr
19	$\alpha$ -Terpineol	1 190	1 189	16.0	tr
20	Cumin aldehyde	1 248	1 242	17.7	0.5 $\pm$ 0.1
21	Caravotanacetone	1 255	1 247	18.2	1.1 $\pm$ 0.2
22	Thujil acetate <iso-3>	1 274	1 270	19.0	0.1 $\pm$ 0.0
23	Sabinyl acetate- <i>trans</i> (IPP vs acetyl)	1 291	1 291	19.4	0.1 $\pm$ 0.0
24	Cymen-7-ol < para >	1 295	1 291	19.5	0.1 $\pm$ 0.0
25	Carvyl acetate <cis >	1 370	1 368	24.0	0.2 $\pm$ 0.0
26	Curcumene-<ar->	1 481	1 481	28.6	tr
$\Sigma$					<b>97.9</b>

Results are expressed as relative percentage in the mixture.

<sup>a</sup>Compounds identified by comparison of their RI (retention indices) and mass spectra with literature data, the MS library (NBS 75K, NIST98), and a spectra library built up from pure substances and components of known oils.

<sup>b</sup>Experimental RI on HP5 MS capillary column in reference to C7-C24 n-alkanes.

<sup>c</sup>Literature data.<sup>16-18</sup>

<sup>d</sup>Compounds listed in order of elution, t<sub>R</sub> (min).

<sup>e</sup>Percentage peak area of EO components as means of 2 determinations  $\pm$  standard deviation.

<sup>f</sup>tr: traces (<0.1%).

**Table 2.** Phenolic Compounds in *Acantholippia salsoloides* Polar Extracts.

Compound	Extract composition <sup>a, b</sup>	
	HE	WE
1 Protocatechuic acid	44.1 ± 0.4 (0.1)	531.2 ± 0.4 (0.8)
2 Catechin	818.0 ± 41.8 (1.3)	1766.0 ± 15.4 (2.6)
3 Phenolic acid derivative 1	303.5 ± 0.8 (0.5)	863.6 ± 19.1 (1.3)
4 Phenolic acid derivative 2	269.8 ± 0.1 (0.4)	960.5 ± 7.2 (1.4)
5 Eriodictyol-7- <i>O</i> -glucoside	392.8 ± 2.6 (0.6)	177.9 ± 0.4 (0.3)
6 Luteolin-8- <i>C</i> -glucoside	678.6 ± 4.5 (1.1)	888.4 ± 4.9 (1.3)
7 Isoferulic acid	29746.5 ± 22.2 (46.6)	30555.3 ± 21.7 (44.8)
8 Apigenin 8- <i>C</i> -glucoside	1528.7 ± 16.4 (2.4)	293.2 ± 9.9 (0.4)
9 Luteolin-6- <i>C</i> -glucoside	221.7 ± 0.2 (0.3)	232.5 ± 2.9 (0.3)
10 Luteolin-3',7-di- <i>O</i> -glucoside	176.7 ± 0.2 (0.3)	123.1 ± 1.5 (0.2)
11 Luteolin derivative (1 + 2)	3925.6 ± 35.1 (6.2)	4763.8 ± 45.0 (7.0)
12 Phenolic acid derivative 3	697.8 ± 4.3 (1.1)	4409.1 ± 8.3 (6.5)
13 Apigenin-6- <i>C</i> -glucoside	3757.3 ± 40.3 (5.9)	1810.6 ± 2.2 (2.7)
14 Luteolin derivative (3 + 4 + 5)	4820.7 ± 19.2 (7.6)	4992.3 ± 13.1 (7.3)
15 Luteolin-7- <i>O</i> -glucoside	11517.9 ± 29.3 (18.1)	10984.8 ± 13.9 (16.1)
16 Luteolin-4'- <i>O</i> -glucoside	251.2 ± 0.4 (0.4)	918.6 ± 2.8 (1.3)
17 Phenolic acid derivative 4	1520.8 ± 1.5 (2.4)	1068.6 ± 2.3 (1.6)
18 Luteolin derivative (6)	1751.8 ± 0.6 (2.7)	1366.1 ± 7.6 (2.0)
19 Luteolin derivative (7 + 8)	1116.2 ± 10.6 (1.7)	1304.9 ± 3.6 (1.9)
20 Luteolin	112.8 ± 0.6 (0.2)	102.9 ± 0.2 (0.2)
21 Apigenin	140.1 ± 0.2 (0.2)	45.58 ± 0.8 (0.1)
Σ	<b>63792.5</b>	<b>68159.0</b>

HE, hydroethanolic extract; WE, decoction extract.

Σ, sum of the determined phenolic compounds (mg/kg or µg/g).

<sup>a</sup>Composition of extracts as mean ± standard deviation of three assays (mg/kg or µg/g).

<sup>b</sup>Relative percentage of compounds in brackets.

profile but showed different compositions. With respect to flavonoids, cynaroside (luteolin-7-*O*-glucoside) (**15**) was the most abundant in HE and WE, followed mainly by several apigenin derivatives and luteolin derivatives. Regarding phenolic acids, they corresponded to ca. 51% and 56% of the phenolic contents in HE and WE, respectively. Isoferulic acid (**7**) was the major of both extracts (Table 2). The phenolic contents ranged between 63792.5 and 68159.0 µg/g of dried extract for HE and WE (Table 2). The recovery of phytochemicals was 1651.6 and 1641.3 µg/g of plant material from HE and WE.

The antibacterial activity of EO and polar extracts was investigated herein; minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values were determined against 6 pathogenic bacteria (Table 3). Gram-positive strains were more sensitive to rica-rica extracts than Gram-negative ones, mainly with HE; similar results were previously reported in other studies.<sup>3,4,15,19</sup> EO and extracts of rica-rica evidenced higher activity against *Staphylococcus aureus* and *Micrococcus luteus* (Table 3).

All extracts revealed to have bactericidal effect on *S. aureus*, but only polar extracts showed bactericidal action on *M. luteus* and *Pseudomonas aeruginosa*. On the other hand, EO showed bactericidal action against *Salmonella typhimurium*. For comparison, MIC and MLC were determined for HE against *S. aureus* ATCC 25923 (Table 3). Gentamicin, MIC = 0.01 mg/mL and MLC = 0.03 mg/mL, was used as control.

*A. salsoloides* shares with *A. deserticola* the identity and percentage order of the major components of EO.<sup>20</sup> Previously, Sampietro et al.<sup>11</sup> attributed a good antimicrobial activity of *A. deserticola* EO to β-thujone and *trans*-sabinyl acetate.<sup>11</sup> In another study, Lima et al.<sup>19</sup> reported promising activity of *A. seriphioides* EO against clinical bacterial strains and yeasts<sup>21</sup>; this oil contained mainly thymol, *p*-cymene, γ-terpinene and carvacrol.

Regarding polar extracts, the antifungal potential of HEs of *A. salsoloides* growing in Catamarca was previously screened with other extremophiles from the Argentine Puna<sup>13</sup>; rica-rica did not show the best

**Table 3.** Antibacterial Activity (MIC and MLC) of *Acantholippia salsoloides* Extracts (mg/mL) and Essential Oil (mL/mL).<sup>a</sup>

Strain	HE		WE		EO	
	MIC	MLC	MIC	MLC	MIC	MLC
<i>Staphylococcus aureus</i> ATCC 25923	0.6	5.0	-	-	-	-
<i>S. aureus</i> ATCC 20231	0.3 - 0.6	5.0	2.5	5.0	7.8	62.5
<i>Micrococcus luteus</i> ATCC 20030	1.3	5.0	2.5	10.0	15.6	>62.5
<i>Bacillus cereus</i> ATCC 31	2.5	10.0	2.5	>10.0	15.6	>62.5
<i>Escherichia coli</i> ATCC 30083	10.0	>10.0	10.0	>10.0	31.3	>62.5
<i>Salmonella typhimurium</i> ATCC 43971	10.0	>10.0	10.0	>10.0	31.3	62.5
<i>Pseudomonas aeruginosa</i> ATCC 50071	10.0	10.0	10.0	10.0	62.5	>62.5

<sup>a</sup>MIC, minimum inhibitory concentration; MLC, minimum lethal concentration. Results are from 3 independent experiments performed in duplicate. Strong activity is for MIC = 0.05-0.5 mg/mL, moderate activity for MIC = 0.6-1.5 mg/mL, and weak activity above 1.5 mg/mL.<sup>15</sup>

performance against postharvest molds. The HEs of *A. punensis* showed promising inhibitory activity against some pathogenic bacteria; these results could be related to the use of this plant as a remedy against urinary and intestinal infections.<sup>22</sup>

The antimicrobial activity depends not only on the amount of specific compounds in EO and extracts, but also on the type of compounds in the phytocomplex.<sup>12</sup> The antibacterial properties of *A. salsoloides* growing in Jujuy could be associated with the high content of specific phenolic compounds, terpenes and terpenoids.

The antioxidant activity of HE and WE was tested against DPPH<sup>•</sup>, superoxide (O<sub>2</sub><sup>•-</sup>), and nitric oxide (NO<sup>•</sup>) radicals. *A. salsoloides* extracts have strong scavenging activity against O<sub>2</sub><sup>•-</sup> and DPPH<sup>•</sup>. Previously Celaya et al reported half-maximal inhibitory concentration (IC<sub>50</sub>) values ranging from 64.1 to 68.5 µg/mL against DPPH<sup>•</sup>, for water extracts of rica-rica from different populations.<sup>6</sup> The 2 extracts studied here showed better behavior, WE being more active than HE (Table 4). For the well-known antioxidant butylated hydroxytoluene (BHT), the radical scavenging activity was

comparable with that of the previous cases (Table 4). The analyzed extracts were particularly active against superoxide anion radical (Table 4). HE was slightly more active than WE against NO<sup>•</sup> (Table 4). The results obtained are comparable with the data previously reported for polar extracts of *Satureja parvifolia* and *Aphyllocladus spartioides* growing in the same region.<sup>14,15</sup>

The main phenolic compounds present in rica-rica extracts (cynaroside and isoferulic acid) are found in several medicinal plants and food matrices. These phytochemicals have demonstrated to have several beneficial effects on human health. Cynaroside among other apigenin and luteolin derivatives was previously isolated from *Salvia chloroleuca* (Lamiaceae) and *Nepeta cataria* (Lamiaceae)<sup>25-28</sup>; these flavonoids were also reported in tissues of *Elaeis guineenses* (Arecaceae) with α-glucosidase inhibitory activity, in *Agrimonia pilosa* (Rosaceae) with antioxidant and α-glucosidase inhibitory activity. Isoferulic acid is one of the main phenolic compounds extracted from *Cimicifugae* species (shengma), belonging to the Ranunculaceae family, which has a long and diverse history of medicinal use; it is the major active ingredient of C.

**Table 4.** IC<sub>50</sub> (mg/mL) Values Found in the Antioxidant Activity and α-Glucosidase Assays for HE and WE.

Sample and controls	Scavenging activity			α-Glucosidase inhibition
	DPPH <sup>•</sup>	NO <sup>•</sup>	O <sub>2</sub> <sup>•-</sup>	
HE	43.4 ± 0.6 <sup>c</sup>	206.0 ± 9.5 <sup>a</sup>	33.3 ± 1.1 <sup>a</sup>	216.7 ± 37.2 <sup>a</sup>
WE	35.5 ± 1.1 <sup>b</sup>	229.7 ± 13.8 <sup>a</sup>	27.5 ± 0.4 <sup>a</sup>	1690.1 ± 39.7 <sup>c</sup>
BHT	25.3 ± 1.0 <sup>a</sup>	-	-	-
Acarbose	-	-	-	271.7 ± 21.3 <sup>b</sup>
Luteolin <sup>23,24</sup>	14.2	12.4	-	6.0
Cynaroside <sup>25-28</sup>	-	-	-	8.2
Isoferulic acid <sup>29,30</sup>	4.6	-	13.3	-

BHT, butylated hydroxytoluene; IC<sub>50</sub>, half-maximal inhibitory concentration; HE, hydroethanolic extract; WE, decoction extract.

<sup>a, b, c</sup>Differences between extracts were tested for significance using the one-way analysis of variance with post hoc Tukey's test. Differences were considered significant for *p*-value <0.05.

*heracleifolia*; the pharmacological properties of this medicinal herb include antioxidative, anti-inflammatory, antiviral, and antidiabetic properties, attributed to the high contents of isoferulic acid in *C. heracleifolia* extracts.<sup>29,30</sup>

The potential of *A. salsoloides* HE and WE to inhibit  $\alpha$ -glucosidase tested *in vitro* (Table 4). WE showed moderate action. HE performed well as  $\alpha$ -glucosidase inhibitor, and showed inhibitory effects comparable to acarbose (Table 4). This activity may be associated with the content of isoferulic acid and luteolin derivatives present in HE extracts.<sup>25–30</sup> Besides the determined phenolic compounds, the presence of other metabolites in the HE that might contribute to the observed  $\alpha$ -glucosidase inhibition cannot be ignored.<sup>31–33</sup>

In relation to the volatile fraction, a *trans*-thujonic population of rica-rica was studied here. Thujone is a bicyclic monoterpene ketone that occurs mainly as a mixture of *alpha* (*cis*) and *beta* (*trans*) diastereoisomers in plants such as *Artemisia absinthium* L. (Asteraceae), *Salvia officinalis* L. (Lamiaceae) (sage), *Thuja occidentalis* L. (Cupressaceae), among others.<sup>32–35</sup> Thujone is commonly used as a flavoring substance in several foods and beverages<sup>32,33</sup>; *cis*-thujone has neurotoxic effect in mammals. Despite this, plant extracts containing the mixture are constituents of many dietary supplements and herbal medicinal products in several countries. The neurotoxic action of *cis*-thujone is associated with high levels of oral exposition.<sup>32–35</sup> *A. salsoloides* present minor contents of this isomer; however, further investigations are required to encourage the use of rica-rica extracts in pharmaceutical and food products.<sup>34,35</sup> The stimulating and digestive properties attributed to *A. salsoloides* could be related to the EO profile as well as the phenolic composition. The decoction is in correspondence with the extensive use of rica-rica for medicinal purposes and food preparations, even though the HE was more active.

## Experimental

### Plant Samples

Aerial parts of 12 specimens (100–200 g each one) were collected during the flowering period, April of 2013, in Chucalezna (23° 21' 39.3" S, 65° 19' 20.9" O, 2702 masl), Jujuy Province (Argentina). The plant material was identified by Professor Osvaldo Ahumada (National University of Jujuy, Argentina) and Professor Gustavo Giberti (National University of Buenos Aires, Argentina); a voucher specimen (HN1308) was deposited in the Herbarium of PRONOA (National University of Jujuy, Argentina). The plant material (leaves and flowers) was dried at room temperature for 7 days, ground to powder in a blender (mean particle size <2 mm) and stored at –20°C until required.

### Preparation of Extracts

EO was obtained from 500 g dry material by steam distillation for 2.5 hours using a Clevenger-type apparatus.<sup>15</sup> The

collected oil was dried and stored at 4°C until analysis. The EO content was determined volumetrically on dried weight basis. WE was prepared boiling 5 g of dried material in 100 mL of H<sub>2</sub>O for 10 minutes.<sup>15</sup> The resulting extracts were filtered through a Büchner funnel, frozen, and lyophilized. HE was prepared with 5 g of dried samples, and sonicated at 40°C with 100 mL of ethanol:water (70:30 v:v) for 20 minutes.<sup>15</sup> The obtained extract was evaporated under reduced pressure and kept at –20°C for further analysis. Extractions were carried out in triplicate.

### Analysis of the Essential Oil

EO composition was analyzed by gas chromatography mass spectrometry and gas chromatography flame ionization detector using a previously described procedure.<sup>15</sup> The quantification of each compound was performed on the basis of their GC/FID peak areas without the use of response factor corrections.<sup>16–18</sup>

### Analysis of Phenolic Compounds

WE and HE were redissolved in methanol and filtered through a 0.45  $\mu$ m polytetrafluoroethylene membrane. The phenolic compounds were analyzed in an HPLC/DAD (Gilson) using the previously described procedure.<sup>15</sup> Thirty microliters of each polar extract were analyzed with an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0  $\times$  0.46 cm; 5  $\mu$ m, particle size; Waters, Milford, MA) column. The chromatograms were recorded at 280 (tannins), 320 (phenolic acids), and 350 nm (flavonoids). The data were processed in Unipoint System software (Gilson Medical Electronics, Villiers-le-Bel, France). The compounds in each sample were identified by comparing their retention times and UV spectra with the library of spectra. The quantification was achieved by measuring the absorbance recorded in the chromatograms relative to external standards. This procedure was performed in triplicate. Phenolic acid derivatives were quantified as 5-*O*-caffeoylquinic acid; luteolin derivatives were quantified as luteolin.

### Antibacterial Activity

The study included 6 bacterial strains: *S. aureus* ATCC 20231, *B. cereus* ATCC 31, *M. luteus* ATCC 20030, *S. typhimurium* ATCC 43971, *E. coli* ATCC 30083, and *P. aeruginosa* ATCC 50071. The MIC and the MLC were determined employing a previously described procedure<sup>15</sup>; *S. aureus* ATCC 25923 was determined as quality control. The tested concentrations were 62.5  $\mu$ L/mL for the EO and 10.0 mg/mL of dry matter for polar extracts.

### Antioxidant Activity

The scavenging activity against DPPH<sup>•</sup>, O<sub>2</sub><sup>•–</sup>, and <sup>•</sup>NO radicals was evaluated for WE and HE according to Celaya et



al<sup>15</sup> Three independent assays were performed for each radical in triplicate. IC<sub>50</sub> values represent the concentrations that caused 50% activity loss. Statistical analysis was carried out using Graph pad Prism 5 Software (San Diego, CA, USA). For the DPPH<sup>•</sup> scavenging activity, the antioxidant BHT was used as reference compound.

### $\alpha$ -Glucosidase Inhibitory Activity

The effect on  $\alpha$ -glucosidase was assessed using a previously reported procedure.<sup>15</sup>

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